Local Adaptation? — Physiological and Biochemical Responses of four Hazelnut Populations to Drought and Possible Impacts on Tree Nurseries[1]

James Gacheru Wanjiku and Heike Bohne[2]

Abstract

Outplanting performance of trees and shrubs cultivated in tree nurseries is assumed to be better if propagation material is sourced from the designated areas of future growth. However, this requires a local nursery to produce that cultivar, which might reduce the availability of that species. In this study we evaluated drought reactions of 2.5-year-old hazelnut (*Corylus avellana* L.) from four population origins. After container cultivation, plants were subjected to drought by irrigating 25% (fast stress) or 50% (slow stress) of the lost water. Control plants were well irrigated. Depending on stress development and hence stress duration, different physiological (stomatal conductance, predawn water potential, relative water content) and biochemical (glucose, fructose, sucrose, starch, proline) responses to drought were found. Independent of stress development, only few differences among populations were found. These differences were mostly not related to precipitation in their area of origin, suggesting no local adaptation within the ecological range investigated.

Index words: area of origin, conservation act, osmolytes, stress.

Species used in this study: hazelnut (*Corylus avellana* L.).

Significance to the Horticulture Industry

Recommendations and regulations concerning the use of local ecotypes for landscape plants add logistical problems regarding sourcing of liners, and cultivation of plants in the nursery might unnecessarily increase the cost of production. Further, it might hamper landscape management as some areas may not have adequate propagules. Ensuing from our results on drought reactions of four hazelnut populations, such procedures could be eased if the species considered has a wide ecological range and populations come from ecologically similar areas.

Introduction

Commercial nurseries' task is to collect and propagate plant species required to restore and establish landscapes. Recently, demand for locally grown plants has increased due to the notion that local ecotypes of propagules might grow best. However, the challenge on nurseries is obtaining quantities of liners with acceptable quality (Mortlock 2000). When local populations' propagules are not adequate or feasible, use of a non-native population had been recommended, provided they are ecologically and functionally similar (Jones 2013).

Use of native populations has sparked a yet-to-end debate with two perspectives. Ecologically, local ecotypes closely match local conditions, which could enhance survival and performance (Jones et al. 2001). Geneticists support the use of local ecotypes to preserve genetic biodiversity (Leinemann et al. 2013) that might further support adaptation to both biotic and abiotic factors. Contrary, use of native populations may not always guarantee better performance (Schreiber et al. 2013) and might be unfit to restore a landscape when the original conditions have been altered.

In Germany, there is an enduring debate principally because of the Federal Nature Conservation Act § 40 (BNatSchG 2010), which aims at conserving regional genetic structures at the population level (transition period until 2020). The underlying principal assumptions of the act are adaptations to local growing conditions and genetic differences between populations. Accordingly, six officially designated areas of origin referred to as provenances for open landscape (not forestry) plants have thus been demarcated. However, growing conditions differ within such an area on a small scale or are not very different from adjacent areas.

Moreover, rapid climate change is a threat across ecosystems and amplifies the question of using currently locally adapted plant populations (Thomas et al. 2014). Concerning drought, trees and shrubs possess physiological and biochemical mechanisms to cope with such stresses. These includes stomata closure and accumulation of compatible solutes like sugars and proline (Chaves et al. 2003). It could be assumed that these mechanisms ensure adaptation and survival of populations not only within their local nativity but also in ecologically similar areas.

Our study explored physiological and biochemical effects of drought on four populations of hazelnut from Germany. Hazelnut is an ecologically important deciduous landscape plant as it provides food and shelter to many insects, birds and rodents (Mehlenbacher 1991, Tallantire 2002). Leinemann et al. (2013) classified some German populations as genetically different using amplified fragment length polymorphism (AFLP).

We evaluated adaptability of four hazelnut German populations to drought stress under controlled conditions. We also compared physiological responses observed within these four ecotypes to their reactions to drought.

[1]Received for publication February 5, 2016; in revised form May 2, 2016. We appreciate financial support by German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) through Agriculture and Food (BLE) Federal Office, grant number 2810BM003. We thank Dr. N. Kowarsch (BLE, Referat 514, Projektträger Agrarforschung) for his kindness and administrative support in the project. We are indebted to Dr. B. Hosius for providing hazelnut cuttings and their basic information.

[2]Graduate student (jemo20032003@yahoo.com) and Professor (bohne@baum.uni-hannover.de), Institute of Horticultural Production Systems, Section: Woody Plant and Propagation Physiology, Leibniz University of Hannover. Herrenhäuser Str. 2, 30419, Hannover, Germany.

Materials and Methods

Hazelnut cuttings were collected by Leinemann et al. (2013), assisted by local forest research centers in identifying native (presumably autochthonous) populations. From this collection, four populations associated with four German federal states were selected: Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF) (Table 1). The four federal states differ partly in climate as shown in Table 1.

In 2009, cuttings were taken from mother plants and rooted. In spring 2010, they were potted into 3 L (#1) containers using Klasmann-Deilmann Peat TS 4® potting substrate (Klasmann-Deilmann GmbH Georg-Klasmann-Str. 2–10, 49744 Geeste, Germany) premixed with Osmocote® (15% N: 9% P_2O_5:11% K_2O: 2% MgO + trace elements) (Everris NA Inc., Dublin, OH) at a rate equivalent to 0.8 g $N{\cdot}L^{-1}$. After one year, they were re-potted into 5 L containers and re-fertilized as above. All plants were cultivated at Leibniz University, Hannover (52°23'34" N; 9°42'13" E; 53 m (174 ft) above sea level) under the same environment and drip irrigation regimes.

Experimental design. In summer 2012, plants were assigned to control or to drought treatments (slow or fast stress) using a completely randomized design with 6 (BB) and 8 (NDS, NRW and RPF) replicates. The experiment was carried out in a greenhouse with an average temperature of 25 ± 4 C (77 ± 39 F).

All containers with plants were saturated before moving them into the greenhouse where they were weighed separately. Stressed plants were placed on spacers while control plants sat on the table. Every two days, stressed plants were weighed and separately irrigated with either 50% (slow stress) or 25% (fast stress) of the lost weight. The control plants were irrigated twice a day by ebb and flow irrigation. The experiment was terminated when half of the plants in each drought treatment withered. The following parameters were evaluated:

Predawn water potential (WP). At predawn (5:00 to 6:00 a.m.) on every other day, leaf water potential was determined from three randomly chosen plants per treatment per population using a Scholander bomb (PMS Instruments, Corvallis, OR). At the end of each treatment, leaf water potential was determined from all drought plants and from three control plants per population. For each measurement the topmost fully expanded leaf was used.

Relative leaf water content (RWC). The same leaf excised for WP was used to determine RWC. Fresh weight of each leaf (FW) was recorded. Each leaf was soaked for 24 hrs in water at room temperature under darkness after which the saturated weight (SW) was recorded. Leaves were oven dried for 24 hrs at 70 C (158 F) before dry weight (DW) was recorded. Then RWC was calculated as:

$$RWC = [(FW - DW) / (SW - DW)] \times 100.$$

Stomatal conductance (SC). Each day (11 a.m. to noon) SC was measured with a steady-state AP4 Porometer (Delta-T Devices, Cambridge, United Kingdom) from the topmost fully expanded leaf from all plants.

Regeneration. Five additional plants per treatment were included in the drought stress experiment and re-irrigated at the end of the drought period. After re-watering, these plants were transplanted into the field for regeneration under natural precipitation. Regeneration was scored by number of shoots, height and root collar diameter five months after drought treatment (end of autumn).

Sampling at the end of the experiment. From each plant, samples for glucose, fructose, sucrose, starch, and proline analysis were taken from the uppermost leaves (3 leaves per shoot). These samples were microwaved for two minutes to deactivate enzymes, and later dried at 70 C for 72 hrs. Each sample was pulverized to a fine powder before analysis.

Carbohydrates analysis (Microplate (MP) method). About 35 mg of ground material was used to extract soluble GFS (glucose, fructose and sucrose) using 4.5 ml (3 times using 1.5 ml each time, 15 min. each) 80% ethanol in warm water bath. Pellet was saved for starch analysis. Glucose, fructose and sucrose were determined enzymatically as detailed by Zhao et al. (2010).

Starch analysis. The pellet (saved above) was suspended with 0.5 ml NaOH (0.5 M). It was then gelatinized by incubating it at 60 C (140 F) for 30 min in a shaker at 150 rpm. After cooling, water and acetic acid (475 µl and 25 µl, respectively) were added, vortexed and centrifuged (1180 × g) for 5 min. In triplicates, 10 µl supernatant was placed in MP. Starch was hydrolyzed to glucose by adding 20 µl amyloglucosidase enzyme (4.5 mg dissolved in 2 ml citrate buffer for one MP) to the sample then incubating for 30 min at 60 C and gentle shaking after 10 min. Starch was quantified using the glucose assay listed above.

Proline analysis. Approximately 50 mg of ground material was suspended in 1.8 ml sulfosalicylic acid (3%) and

Table 1. Map coordinates, altitude, average rainfall, and average temperature for the four hazelnut populations used in this research[z].

Population	Altitude (m)	Latitude	Longitude	Rainfall (mm)		Air Temp. (C)	
				Summer	Annual	Summer	Annual
Brandenburg	38	52°38′	12°58′	160–180	475– 550	17–18	8.5–9
Niedersachsen	63	52°23′	9°31′	200–240	600– 700	16–17	8.0–9
Nordrhein-Westfalen	115	51°45′	9°22′	180–240	700– 900	16–17	7.0–9
Rheinland-Pfalz	464	50°17′	7°00′	180–240	700–1000	14–17	7.0–9

[z]Air temperatures and rainfall data are 30 years averages (1961–1990) from KlimaatlasBundesrepublik Deutschland: Karte 1.13 and 1.15 (annual and summer temperature); Karte 2.13 and 2.15 (annual and summer rainfall). http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&_windowLabel=T3860013424116972633808 6&_urlType=action&_pageLabel=_dwdwww_klima_umwelt_ueberwachung_deutschland.

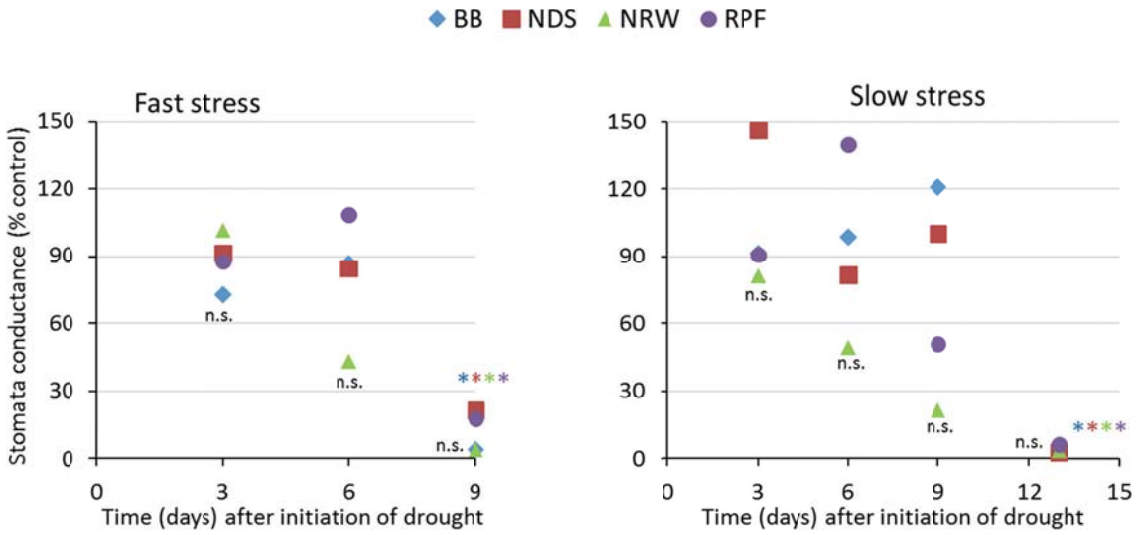

Fig. 1. Stomatal conductance (% of untreated plants) of four populations of container-grown *Corylus avellana* during two drought treatments. Mean; n = 3 during the drought period; n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF) on the last day. ** = significant differences between each population's stress treatment and its control (raw data). n.s. = no significant difference among populations as per Tukey test, p ≤ 0.05.**

incubated on ice for 30 min. The homogenate was vortexed and centrifuged (14462 × g) for 15 min. Thereafter 150 µl of the supernatant was treated with 90 µl acetic acid and 90 µl acid-ninhydrin followed by boiling in a water bath for 45 min. After cooling, 1.5 ml toluene was added and vortexed. The colored toluene phase (200 µl) was pipetted in MP (triplicates) and absorbance at 520 nm was determined using a photometer (VERSAmax® Molecular Device, Sunnyvale, CA). Acid ninhydrin was prepared as described by Bates et al. (1973).

Statistical analysis. Data from all parameters were subjected to multivariate analysis of variance (MANOVA) to test treatment and population main effects or interactions between them. Data was log transformed for normal distribution prior to analyses. Where there were no interactions, treatments and population means, at p ≤ 0.05, were separated by Tukey test. All statistical analyses were performed with R 3.1.3 (R Development Core Team 2014, R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

Drought stress did not influence most physiological parameters during the first six days after drought initiation. During this period, treated plants retained similar stomata conductance (Fig. 1), predawn water potential and relative water content values as those of controls (data not shown). At day 9 (D9) for fast and 13 (D13) for slow stress, over 50% of the treated plants were severely wilted, marking the end of the drought experiment. Only then was stomatal conductance decreased significantly differing compared to the control.

At D9 and D13, control plants had retained high WP (≥ –0.9 MPa) while the treated plants had significantly lower WP, up to –3.2 MPa irrespective of origin (Table 2). Fast stress induced lower WP than slow stress. At D9, RWC of all fast stress treated plants was significantly lower com-

Table 2. Leaves' water potential (WP), relative water content (RWC), glucose, fructose, sucrose, starch, and proline of four container-grown *Corylus avellana* populations at the end of fast stress (D9) and slow stress (D13) treatments[z].

Treatment	Origin	WP (MPa)	RWC (%)	Glucose (% dm)	Fructose (% dm)	Sucrose (% dm)	Starch (% dm)	Proline (µg·g^{-1})
Control	BB	–0.7 ± 0.1Ba	80 ± 04Ba	0.97 ± 0.1Aa	0.88 ± 0.1Aa	4.43 ± 0.3Ca	0.63 ± 0.4Ba	42 ± 19Aa
	NDS	–0.9 ± 0.1Ba	73 ± 04Ba	1.15 ± 0.4Aa	0.88 ± 0.3Aa	4.33 ± 0.6Ba	0.49 ± 0.2Ba	38 ± 13Aa
	NRW	–0.7 ± 0.1Ba	81 ± 02Ba	1.02 ± 0.2Aa	0.83 ± 0.4Aa	4.04 ± 0.9ABa	0.41 ± 0.4Ba	38 ± 24Aa
	RPF	–0.7 ± 0.2Ba	75 ± 04Ba	1.08 ± 0.2Aa	0.98 ± 0.3Aa	4.01 ± 0.6ABa	0.65 ± 0.3Ba	52 ± 13Aa
Slow stress	BB	–2.0 ± 0.9Aa	60 ± 14Aa	1.35 ± 0.2Aa	1.30 ± 0.3Aa	**2.97 ± 0.4Aa**	0.08 ± 0.03Aa	138 ± 35Bab
	NDS	2.1 ± 1.3Aa	72 ± 14ABa	1.44 ± 0.2ABa	1.43 ± 0.2Ba	3.34 ± 0.4Aab	0.07 ± 0.02Aa	**159 ± 47Bb**
	NRW	1.7 ± 0.7Aa	71 ± 08Aa	1.25 ± 0.2Aa	1.30 ± 0.2Ba	3.18 ± 0.3Aab	0.08 ± 0.06Aa	142 ± 48Bab
	RPF	–2.1 ± 1.1Aa	71 ± 14ABa	1.34 ± 0.4ABa	1.22 ± 0.2ABa	**3.73 ± 0.5Ab**	0.07 ± 0.02Aa	**93 ± 26Ba**
Fast stress	BB	3.1 ± 0.2Aa	58 ± 09Aa	1.30 ± 0.5Aa	1.26 ± 0.5Aa	3.70 ± 0.6Ba	0.05 ± 0.02Aa	162 ± 33Ba
	NDS	2.9 ± 0.9Aa	59 ± 11Aa	1.59 ± 0.5Ba	1.52 ± 0.6Ba	4.49 ± 0.7Ba	0.20 ± 0.3Aa	184 ± 64Ba
	NRW	–3.1 ± 0.7Aa	57 ± 09Aa	1.81 ± 0.5Ba	1.39 ± 0.5Ba	4.66 ± 1.3Ba	0.23 ± 0.2ABa	168 ± 45Ba
	RPF	3.2 ± 0.7Aa	55 ± 10Aa	1.63 ± 0.1Ba	1.39 ± 0.2Ba	4.85 ± 0.9Ba	0.06 ± 0.03Aa	193 ± 114Ca

[z]Mean ± SD, n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF). Different letters show significant differences: capital letter among the treatments within a population while small letters among the populations within a treatment.

pared to the control. This was not the case for plants in the slow stress treatment at D13 (Table 2). For all physiological parameters, there were no significant population differences. NRW plants in both slow and fast stress treatment tended to close their stomata more quickly compared to the other populations (Fig. 1).

Drought stress (slow and fast) caused varying effects on glucose, fructose and sucrose in the leaves (Table 2). Slow stress did not affect glucose concentration among the four ecotypes. It however increased fructose concentration in NDS and NRW but not in BB and RPF. Sucrose concentration was decreased in BB and NDS but was not affected by slow stress in NRW and RPF. Fast stress prompted an increase in leaves' glucose and fructose concentration in NDS, NRW and RPF but not in BB. Sucrose concentration in leaves was not affected by fast stress in NDS, NRW and RPF but declined in BB compared to the control. Leaves' starch concentration declined significantly (except NRW in fast stress) compared to the control trees irrespective of drought stress level and origin.

Proline increased in leaves similarly in the two stress treatments (Table 2). In slow stress, RPF had the lowest concentration of proline and differed significantly from NDS.

Combining results from slow and fast stress treatments at the end of drought, decreasing WP significantly decreased SC and RWC and increased proline concentration in all populations. This is supported by significant linear correlations between these three parameters and predawn water potential (Table 3). Starch and GFS were least affected by decreasing water potential. However, in NRW GFS were significantly influenced by WP (Table 3).

After the drought treatments, plants suffered up to 25 cm shoots' dieback, especially plants in fast stress (data not shown). However, they were able to equally regenerate and by the end of the growing season, the differences in height with the control plants were not statistically different (data not shown).

Stomata react to soil and atmospheric induced drought stress. Stomatal closure is often reported as the first drought avoidance mechanism in plants (Harb et al. 2009). In our experiment, this was not demonstrated significantly before the end of the drought stress. Among others, water potential in the guard cells regulates stomatal behavior. Air humidity between 55 to 65% in the greenhouse and water supply (although decreasing per each irrigation) might have minimized water losses of the growing medium. In both drought treatments, the stress signal may have been weak or too short to cause a reaction. In a different experimental setup, Schulze and Küppers (1979) found that short-term changes in leaf water potential of *Corylus avellana* L. had little influence on stomatal conductance. This response was also found for *Prunus armeniaca* L. (Schulze et al. 1974) and attributed to an optimization of carbon gain versus water loss. As demonstrated in this experiment with fast stress development, further decreasing the water supply led to a sudden closure of stomata, but plants leaves' had reached the turgor loss point. The tested populations did not differ in their response to the abrupt water deficit, although BB comes from a low precipitation area.

Although stomata closed late, slow-stress plants maintained similar RWC to the control, while WP declined significantly. Lower WP can be achieved by decreasing water content, osmotic adjustment and/or elasticity of the cell wall (Ɛ) (Bartlett et al. 2012). In slow stress, osmotic adjustment was low (Table 2). The effect of Ɛ is discussed controversially in literature. According to Bowman and Robert (1985), high Ɛ (decreasing cell wall elasticity) results in a quick decrease in WP for a given change in water content, which favors further water uptake from a drying soil. This was supported by Savé et al. (1993) with strawberry (*Fragaria* spp.) subjected to mild drought stress and could also be a reason for our results. It can only be assumed that our fast stress treatment did not allow such adaptions. Both RWC and WP were more affected in fast than in slow stress. Loss of water through stomata and passive/active accumulation of solutes led to a very low RWC (55 to 59%). Read et al. (2010) reported for deciduous *Nothofagus cunninghamii* (Hook.) Oerst. that an RWC of 55% caused leaf damage as measured by electrolyte leakage. Although thresholds for RWC seem to be species dependent (Lawlor and Cornic 2002, Dichio et al. 2006), this range applies also for the leaves of hazelnut in our fast stress treatment.

The experimental setup resulted in different possibilities of physiological reactions to drought. While slow stress probably allowed adjustments in cell wall elasticity, fast stress did not. But independent of stress development, there were no differences in population's physiological reactions, suggesting a range of potentials against any assumed local adaptation.

Osmotic adjustment in terms of accumulating compatible solutes like proline, glucose, fructose and sucrose is reported as a mechanism for plants to tolerate drought (Chaves et al. 2003). Differences related to stress development and among populations (although not always) were found for these compounds. Proline concentration increased the most, mainly increasing independently of the stress level and (with one exception) populations' origin. Among biochemical reactions, only proline concentration strongly correlated with water potential (Table 3). This agrees with literature where proline is reported to increase with water stress (Guo et al. 2010, Boussadia et al. 2013). The reaction of GFS differed for slow and fast stress. While there were few increases in glucose and fructose at the end of slow stress, there were significant glucose and fructose increases due to fast stress

Table 3. Pearson's correlation coefficients obtained by relating predawn water potential (WP) with various parameters of four container-grown *Corylus avellana* populations[z].

Year	Origin	SC	RWC	Glucose	Fructose	Sucrose	Starch	Proline
2012	BB	**0.65**	**0.79**	–0.16	–0.22	0.11	0.56	**–0.83**
	NDS	**0.52**	**0.83**	–0.37	–0.33	–0.03	0.18	**–0.78**
	NRW	**0.57**	**0.91**	**–0.72**	**–0.42**	**–0.58**	0.19	**–0.66**
	RPF	**0.45**	**0.61**	–0.25	–0.33	–0.36	0.58	**–0.72**

[z]Significant correlations ($p \leq 0.05$) are given in bold. n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF).

compared to the control, except for population BB. Concerning sucrose for slow and fast stress, BB decreased its concentration significantly while this was not the case for the other populations (except NDS slow stress). However, the sucrose concentration neither increased in these populations. The stress treatments also had different stress duration. Boussadia et al. (2013), investigating two cultivars, found glucose and fructose concentration partially increased after 10 days of drought and decreased after 20 days compared to the control. Either the longer drought duration prevented plants from sustaining higher concentration of these osmolytes (glucose, fructose, and sucrose) or they were used up for other sugars or sugar alcohols synthesis like mannitol and inositol. The latter was shown by Boussadia et al. (2013) for two cultivars of olive (*Olea europaea* L.), but even the cultivars tested reacted differently. From our data we could not detect if the decrease of a single sugar was due to metabolism, resulting in possible other osmolytes. Among the populations, only NRW GFS concentration was significantly correlated with WP (Table 3). The reason remains open but cannot be attributed to climate, since the one for NRW is not much different from NDS and RPF.

Starch degrading enzymes are often reported to increase with water stress (Chaves et al. 2003, Harb et al. 2010). These enzymes may be associated with a decline in starch concentration in both the slow and fast stress treatments.

In summary, as for physiological reactions, results for GFS were only partially different within the populations tested. Moreover, the few differences found could not be linked to the population's climatic conditions (Table 1), if at all for BB, coming from a low precipitation area compared to others. This agrees with the findings from Peuke et al. (2002) for beech (*Fagus* spp.) seedlings, who found no distinct influence of provenance on drought-related physiological and biochemical parameters, and no consistent relation to areas of origins' rainfall amount. Additionally, the few differences among our hazelnut populations during drought were not reflected in regeneration, which was equally good for all populations.

Mainly insignificant differences found in the drought reactions of the investigated populations suggest no local adaptation. This attests that hazelnut is adapted to wide ecological conditions (Mehlenbacher 1991) attributed to common ancestry (Willis 1996). The genetic differentiations reported by Leinemann et al. (2013) were not featured by the species reaction to drought. Consequently, if only adaptation to growing conditions is considered, for a species with wide ecological adaptations the efforts of discrete sourcing of propagation material and cultivation of plants in the nursery concerning the area of origin may be alleviated. This would facilitate nursery cultivation and supply of adapted species, which might not be available if a certain area of origin of the species is prescribed.

Literature Cited

Bartlett, M.K., C. Scoffoni, and L. Sack. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol. Lett. 15:393–405.

Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39:205–207.

Boussadia, O., A. Bchir, K. Steppe, M.-C. Van Labeke, R. Lemeur, and M. Braham. 2013. Active and passive osmotic adjustment in olive tree leaves during drought stress. Euro. Sci. J. 9:423–439.

Bowman, W.D. and S.W. Roberts. 1985. Seasonal changes in tissue elasticity in chaparral shrubs. Physiol. Planta. 65:233–236.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. Funct. Plant Biol. 30:239–264.

Dichio, B., C. Xiloyannis, A. Sofo, and G. Montanaro. 2006. Osmotic regulation in leaves and roots of olive trees during a water deficit and rewatering. Tree Physiol. 26:179–185.

Federal Nature Conservation Act §40 (BNatSchG/Gesetz über Naturschutz und Landschaftspflege) 2010. Issued by the German government. Bundesnaturschutzgesetz vom 29.7.2009, in Kraft getreten am 1.3.2010. BGBI I: 2542.

Guo, X., X. Zhang, and Z. Huang. 2010. Drought tolerance in three hybrid poplar clones submitted to different watering regimes. J. Plant Ecol. 3:79–87.

Harb, A., A. Krishnan, M.M.R. Ambavaram, and A. Pereira. 2010. Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol. 154:1254–1271.

Jones, A.T., M.J. Hayes, and N.R. Sackville Hamilton. 2001. The effect of provenance on the performance of *Crataegus monogyna* in hedges. J. Appl. Ecol. 38:952–962.

Jones, T.A. 2013. When local isn't best. Evol. Appl. 6:1109–1118.

Lawlor, D.W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell and Environ. 25:275–294.

Leinemann, L., W. Steiner, B. Hosius, O. Kuchma, W. Arenhövel, B. Fussi, B. Haase, R. Kätzel, M. Rogge, and R. Finkeldey. 2013. Genetic variation of chloroplast and nuclear markers in natural populations of hazelnut (*Corylus avellana* L.) in Germany. Plant Syst. and Evol. 299:369–378.

Mehlenbacher, S.A. 1991. Hazelnuts (*Corylus*) Genetic resources of temperate fruit and nut. Acta Hort. 290:791–836.

Mortlock, W. 2000. Local seed for revegetation. Ecol Manage. Restor. 1:93–101.

Peuke, A.D., C. Schraml, W. Hartung, and H. Rennenberg. 2002. Identification of drought-sensitive beech ecotypes by physiological parameters. New Phytol. 154:373–387.

Read, J., R.S. Hill, and G.S. Hope. 2010. Contrasting responses to water deficits of *Nothofagus* species from tropical New Guinea and high-latitude temperate forests: Can rainfall regimes constrain latitudinal range? J. Biogeogr. 37:1962–1976.

Savé, R., J. Peñuelas, O. Marfà, and L. Serrano. 1993. Changes in leaf osmotic and elastic properties and canopy structure of strawberry under mild water stress. HortSci. 28:925–927.

Schreiber, S.G., C. Ding, A. Hamann, U.G. Hacke, B.R. Thomas, and J.S. Brouard. 2013. Frost hardiness vs. growth performance in trembling aspen: An experimental test of assisted migration. J. Appl. Ecol. 50:939–949.

Schulze, E.-D., O.L. Lange, M. Evenari, L. Kappen, and U. Buschbom. 1974. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. I. A simulation of the daily course of stomatal resistance. Oecologia 17:159–170.

Schulze, E.-D. and M. Küppers. 1979. Short-term and long-term effects of plant water deficits on stomatal response to humidity in *Corylus avellana* L. Planta 146:319–326.

Tallantire, P.A. 2002. The early-Holocene spread of hazel (*Corylus avellana* L.) in Europe north and west of the Alps: An ecological hypothesis. The Holocene 12:81–96.

Thomas, E., R. Jalonen, J. Loo, D. Boshier, L. Gallo, S. Cavers, S. Bordács, P. Smith, and M. Bozzano. 2014. Genetic considerations in ecosystem restoration using native tree species. For. Ecol. and Manag. 333:66–75.

Willis, K.J. 1996. Where did all the flowers go? The fate of temperate European flora during glacial periods. Elsevier Sci. 20:110–114.

Zhao, D., C.T. MacKown, P.J. Starks, and B.K. Kindiger. 2010. Rapid analysis of nonstructural carbohydrate components in grass forage using microplate enzymatic assays. Crop Sci. 50:1537–1545.